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Potential role of blood biomarkers in the management of nontraumatic intracerebral hemorrhage

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Abstract: **BACKGROUND:** Intracerebral hemorrhage (ICH), a subtype of stroke associated with high mortality and disability, accounts for 13% of all strokes. Basic and clinical research has contributed to our understanding of the complex pathophysiology of neuronal injury in ICH. Outcome rates, however, remain stable, and questions regarding acute management of ICH remain unanswered. Newer research is aiming at matching measured levels of serum proteins, enzymes, or cells to different stages of brain damage, suggesting that blood biomarkers may assist in acute diagnosis, therapeutic decisions, and prognostication. This paper provides an overview on the most promising blood biomarkers and their potential role in the diagnosis and management of spontaneous ICH. **SUMMARY:** Information was collected from studies, reviews, and guidelines listed in PubMed up to November 2013 on blood biomarkers of nontraumatic ICH in humans. We describe the potential role and limitations of GFAP, S100B/RAGE, and ApoC-III as diagnostic biomarkers, -Amyloid as a biomarker for etiological classification, and 27 biomarkers for prognosis of mortality and functional outcome. Within the group of prognostic markers we discuss markers involved in coagulation processes (e.g., D-Dimers), neuroendocrine markers (e.g., copeptin), systemic metabolic markers (e.g., blood glucose levels), markers of inflammation (e.g., IL-6), as well as growth factors (e.g., VEGF), and others (e.g., glutamate). Some of those blood biomarkers are agents of pathologic processes associated with hemorrhagic stroke but also other diseases, whereas others play more distinct pathophysiological roles and help in understanding the basic mechanisms of brain damage and/or recovery in ICH. **KEY MESSAGES:** Numerous blood biomarkers are associated with different pathophysiological pathways in ICH, and some of them promise to be useful in the management of ICH, eventually contributing additional information to current tools for diagnosis, therapy monitoring, risk stratification, or intervention. Up to date, however, no blood biomarker of ICH has been studied sufficiently to find its way into clinical routine yet; well-designed, large-scale, clinical studies addressing relevant clinical questions are needed. We suggest that the effectiveness of biomarker research in ICH might be improved by international cooperation and shared resources for large validation studies, such as provided by the consortium on stroke biomarker research (<http://stroke-biomarkers.com/page.php?title=Resources>).

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Potential Role of Blood Biomarkers in the Management of Nontraumatic Intracerebral Hemorrhage

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Key Words

Blood biomarker · Intracerebral hemorrhage · Management · Stroke

Abstract

Background: Intracerebral hemorrhage (ICH), a subtype of stroke associated with high mortality and disability, accounts for 13% of all strokes. Basic and clinical research has contributed to our understanding of the complex pathophysiology of neuronal injury in ICH. Outcome rates, however, remain stable, and questions regarding acute management of ICH remain unanswered. Newer research is aiming at matching measured levels of serum proteins, enzymes, or cells to different stages of brain damage, suggesting that blood biomarkers may assist in acute diagnosis, therapeutic decisions, and prognostication. This paper provides an overview on the most promising blood biomarkers and their potential role in the diagnosis and management of spontaneous ICH. **Summary:** Information was collected from studies, reviews, and guidelines listed in PubMed up to November 2013 on blood biomarkers of nontraumatic ICH in humans. We describe the potential role and limitations of GFAP, S100B/RAGE, and ApoC-III as diagnostic biomarkers, β -Amyloid as a biomarker for etiological classification, and 27 bio-

markers for prognosis of mortality and functional outcome. Within the group of prognostic markers we discuss markers involved in coagulation processes (e.g., D-Dimers), neuroendocrine markers (e.g., copeptin), systemic metabolic markers (e.g., blood glucose levels), markers of inflammation (e.g., IL-6), as well as growth factors (e.g., VEGF), and others (e.g., glutamate). Some of those blood biomarkers are agents of pathologic processes associated with hemorrhagic stroke but also other diseases, whereas others play more distinct pathophysiological roles and help in understanding the basic mechanisms of brain damage and/or recovery in ICH.

Key Messages: Numerous blood biomarkers are associated with different pathophysiological pathways in ICH, and some of them promise to be useful in the management of ICH, eventually contributing additional information to current tools for diagnosis, therapy monitoring, risk stratification, or intervention. Up to date, however, no blood biomarker of ICH has been studied sufficiently to find its way into clinical routine yet; well-designed, large-scale, clinical studies addressing relevant clinical questions are needed. We suggest that the effectiveness of biomarker research in ICH might be improved by international cooperation and shared resources for large validation studies, such as provided by the consortium on stroke biomarker research (<http://stroke-biomarkers.com/page.php?title=Resources>).

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Introduction

Epidemiology of ICH

Stroke annually brings 15 million patients into emergency rooms worldwide, of which 5.5 million die and another 5 million are left permanently disabled [1]. Stroke is primarily categorized into ischemic (approximately 87%) and hemorrhagic (approximately 13%, including subarachnoid hemorrhages) stroke [2].

Spontaneous intracerebral hemorrhage (ICH) is associated with poorer outcome compared to ischemic stroke (IS), with 30-day mortality rates of 37–52% [2] and only 20% of survivors returning to independent function at 6 months [3]. These rates have not changed over the past 20 years [4].

While high blood pressure used to account for 80–90% of ICH, today this number has decreased to 50%, presumably as a result of efficient hypertension management [5] and an increasing accuracy in the diagnosis of other etiologies because computed tomography (CT) and especially magnetic resonance imaging (MRI) are more widely available. The gap therefore is being filled up by an increasing number of long-term anticoagulation-related ICH (17% of ICH) [6], for example, on the basis of vascular malformations, and especially cerebral microbleeds [7], which are generally associated with larger hemorrhage size and higher mortality. The third etiological subcategory, accounting for 20% of ICH, is the deposition of amyloid protein in cerebral arteries (cerebral amyloid angiopathy, CAA), causing the blood vessels to leak more easily [5].

Pathophysiology of ICH

Advances in neuroimaging and animal models have improved our understanding of the pathophysiology of ICH on macro-, molecular, and genetic levels [8]. By analyzing the cascade of brain damage after ICH, targets for new therapies and future biomarkers can be identified. Most current theories imply a two-phase model of neuronal injury with an early mechanical phase and a subacute phase of inflammation and edema formation as response to the hemorrhage [9], as well as the parallel activation of the neuroendocrine stress axis.

Primary Phase: Mechanical Damage

Within 60 min after the insult, the blood released into the brain parenchyma leads to the compression of anatomical structures and increased intracranial pressure, potentially leading to brain herniation [10], decreased blood flow, and direct mechanical destruction of axons and glial cells [11] (fig. 1).

Secondary Phase: Physiological Response to Hematoma

The body reacts to the bleeding and rise of intracranial pressure with different parallel mechanisms that accelerate themselves and each other, increasingly compounding the neurological injury (fig. 2).

One of the first responses to acute bleeding is the stress-related activation of the hypothalamo-pituitary-adrenal (HPA)-axis as well as the sympathetic nervous system (SNS) [12], both of which act systemically. Locally, glutamate is released by necrotic perihematomal neurons and by the ruptured vessels, leading to the accumulation of oxidative byproducts, further necrosis of neighboring cells, and early cytotoxic edema formation [13].

Within the first two days, hemostatic mechanisms occur to limit the bleeding. Thrombin is released and affects a range of cells in the surrounding penumbra, creating inflammatory, neurotoxic conditions. Thrombin activates microglial cells to release inflammatory mediators, causes proliferation of mesenchymal cells and formation of scar tissue, and leads to endothelial cell dysfunction, resulting in hyperpermeability, disruption of blood brain barrier (BBB), and vasogenic edema formation [14].

After several days, iron, hemoglobin, and free radicals are released into the hemorrhagic area, following the lysis of erythrocytes and clot components as part of the thrombin response, causing further inflammation and late edema formation [15].

The last mechanism resulting from intracerebral inflammation and late edema is the activation of the complement system. As C5a and C3a penetrate the neuronal tissue, local glia, mast cells, and leukocytes are activated, followed by the additional damage of perihematomal neurons, glia, and blood vessels, and further cell lysis and inflammation [16].

Management of ICH

Modern ICH management including accurate diagnosis, effective treatment, prediction of progression, response to therapy, and prognostication remains challenging. Management decisions are mainly based on neuroimaging information such as hematoma volume, location, and the presence and quantity of intraventricular bleeding, and clinical information such as age and the Glasgow coma scale (GCS). These features are all represented in the Hemphill ICH score [17]. As ICH is a medical emergency, it would be beneficial to have a diagnostic tool applicable in pre-hospital diagnosis, early sub-typing, and risk stratification. Blood biomarker (BBM) research may be helpful.

potheses, searching pathophysiologic mechanisms of ICH for in blood measurable, involved agents.

Newer approaches based on genetic and molecular research are fairly unbiased discovery processes. Gene expression profiling has generated diverse insights on RNA expression and protein and metabolite production, depicting changes in expression, induction, and function of genes in individuals with stroke. The advantage of such approaches is that they generate a number of potential markers of interest, but the difficulty is that the utility of each individual marker remains uncertain until further studies can validate the results [19]. Proteomics and metabolomics are new techniques to systematically scan blood from cases and controls for differentially expressed proteins and molecules (lipids, sugars, nucleotides, amino acids), potentially revealing new disease-related particles [20]. These methods, in opposition to genetic profiling, respond more strongly to environmental influences and thus are dynamic in reflecting current physiologic states [21]. Genetic and molecular technologies belong to the very first discovery phase of BBM research as an enormous flood of information is generated, which needs versatile elaborate analysis to select those agents possibly meaningful for further studies.

While we recognize that cerebrospinal fluid biomarkers may have certain advantages over blood biomarkers, we focus this review on blood biomarkers alone because they are more readily measured repeatedly; there are a large number of studies addressing blood biomarkers, and space is limited.

Search Methods

A comprehensive literature search was conducted on PubMed up to November 2013, including citation and reference tracking to identify the blood biomarkers cited in this review. The search terms employed were: 'ICH', 'intracerebral h(a)emorrhage/h(a)ematoma', 'h(a)emorrhagic stroke' + 'biomarker(s)' + 'management', 'outcome', 'prognosis', 'diagnosis', and 'etiology'. With this retrieval strategy a total of 186 manuscripts was yielded.

Clinical Implementation of ICH Blood Biomarkers

Diagnostic Blood Biomarkers

Diagnostic BBMs should be able to reliably distinguish between patients suffering from ICH, IS, and stroke mimics such as seizures, migraines, syncope, or metabolic conditions [22], enabling rapid medical interventions. This information is required shortly after symptom onset be-

cause early neurological damage within the first few hours after stroke is strongly associated with poor long-term outcome [23]. BBMs differentiating ICH from IS already in the ambulance might be especially useful to start blood pressure lowering therapies in a very early stage (as suggested by the results of the INTERACT2 trial [24]).

Etiologic Blood Biomarkers

Etiologic BBMs should allow differentiation between bleeding based on hypertension, long-term anticoagulation, CAA or other more infrequent causes. Such BBMs could increase the quality of rapid specific treatment, which differs according to the pathogenesis (blood pressure control, immediate cessation of anticoagulant therapy, hemostatic therapy depending on the drug used, and/or surgical hematoma evacuation). Etiologic BBMs should be found in blood sampled at admission as only early information can lead to early therapy.

Blood Biomarkers of Prognosis

Prognostic BBMs have the potential to predict end-points like complications, mortality, or poor functional outcome, and therefore may enable preventive and therapeutic interventions [25]. Moreover, to later allow the integration of a BBM into the package of clinical instruments, its ability to depict specific causes of worsening (e.g., infection, pneumonia, epileptic seizures, hematoma growth), and prognosis-related situations that allow specific intervention, is crucial. For example, early time to surgery (excluding emergency decompression) was shown to improve mortality rates but also increase the risk of rebleeding. Sixty percent of ICH patients are at a low risk for further bleeding, and might therefore qualify for surgery, whereas patients with a high risk of rebleeding might benefit more from medical intervention only [26–28]. A BBM reliably discriminating the low risk from the high risk-rebleeding group would be very useful for the intervention triage. Depending on the time-point of blood withdrawal, prognostic BBMs could also enable early risk stratification, emergency health care decisions, and late interventions, such as rehabilitation and follow-up care. As prognostic BBMs correlate in an either protective or damaging way with disease progression, they might also help in identifying new therapeutic targets.

Blood Biomarkers as Surrogates

Surrogate markers are markers that substitute for true end points. This correlation needs to show a robust stability across different beneficial as well as aggravating interventions and alterations of circumstances, before a change

of the biomarker might actually reliably predict the clinical outcome (in place of the clinical outcome itself) and therefore account as surrogate [29]. This high bar is set to prevent misleading information regarding the true end point by a 'surrogate' and is a tall order for any blood test. None of the BBMs mentioned below serves as such a good marker of change in disease status that it could be used in place of other measures (yet).

The most advanced BBM examples for each category are summarized in table 1.

Selection of Blood Biomarkers in ICH

Blood Biomarkers for Diagnosis

GFAP

Glial fibrillary acidic protein (GFAP) is an intermediate filament expressed by astrocytes and ependymal cells. After ICH it can be found in serum of patients due to necrotic brain cell destruction and BBB disruption. Low concentrations of GFAP are found in the blood of healthy controls; [30] it is increased after IS, but the highest concentrations are found in early ICH patients [31].

In a recent multicenter cohort study including 205 patients diagnosed with either IS, ICH, or stroke mimics, plasma GFAP on admission had a sensitivity of 84% and specificity of 96% for differentiating ICH from IS and stroke mimics (AUC 0.915, 95% CI 0.847–0.982) [32].

Since this study only included patients with hemispheric stroke symptoms, the results cannot be generalized to all ICH patients. This also may have led to a higher sensitivity. Furthermore, only few mimics were included, potentially causing artificial increase of specificity.

S100B/RAGE

The transmembrane Receptor for Advanced Glycation Endproducts (RAGE) is a multiligand member of the immunoglobulin superfamily, which plays a major role in both early development and progression of atherosclerosis and vascular inflammation [33]. S100B is a brain-specific, calcium-binding protein and ligand for RAGE, expressed and released mainly by astrocytes [34]. S100B/RAGE interactions might show different expression courses in inflammatory or hypoxic brain damage.

A large study recently showed the ability of the S100B/RAGE BBM panel to distinguish between IS and ICH in a sample of 915 stroke patients (AUC 0.76) [35]. In blood sampled within 6 h from symptom onset, S100B was elevated, whereas sRAGE was lower in ICH patients compared to patients with IS. The additive value of the panel

to the AUC when added to the model using only clinical signs was significant (AUC 0.84 vs. AUC 0.77) and even stronger when measured earlier (<3 h from stroke; AUC 0.81 vs. AUC 0.76) [35].

Although promising, the dynamics of sRAGE in stroke is not yet clearly understood. The results need validation in larger cohort studies including different settings, stroke mimics, and additional BBMs.

ApoC- III

ApoC-III is a member of the apolipoprotein family expressed by the liver. It is found mainly in very-low-density lipoprotein (VLDL) particles and is associated with cardiovascular risk.

In a small case-control study that measured ApoC-III expression in stroke patients within 6 h from symptom onset, a relative overexpression of ApoC-III was found in IS compared to ICH patients. ApoC-III levels allowed differentiation of ICH from IS with 94 sensitivity and 87% specificity and distinguished ICH from healthy controls with 100% sensitivity and 67% specificity [36].

So far, ApoC-III seems to be an interesting marker to distinguish ICH from IS, but the sample size was small and data was not collected prospectively. A larger validation study should also include stroke mimics.

Blood Biomarkers for Etiological Classification

β -Amyloid

β -Amyloid (β A) is a protein pathologically deposited in smaller arteries of the brain of patients suffering from CAA, causing them to leak or rupture more easily [37].

By comparing plasma of CAA-related ICH patients to healthy controls, it was shown that β A was significantly higher in CAA cases than in controls. β A levels even differentiated between patients with probable CAA and those diagnosed with possible CAA ($p < 0.015$) or controls ($p < 0.005$) [38]. The development of a β A screening method for CAA would be particularly useful, as diagnosis of CAA prior to hemorrhage is not yet possible [39]. Confirmation in a prospective multicenter study is indispensable, as the sample size was small and a previous study showed contradicting results [40].

Blood Biomarkers for Prognosis and as Pharmacological Targets

MMP-9

Matrix metalloproteinase 9 (MMP-9) is a protease induced by thrombin and blood, which increases capillary permeability, disrupts BBB, and is neurotoxic by degrading the endothelial basal lamina and extracellular

Table 1. Blood biomarkers in the management of ICH

Blood biomarkers in the acute management of ICH					
blood biomarker	description	potential clinical application	study reference	size	study design time points
Diagnostic markers					
GFAP	Highly brain-specific intermediate filament protein predominantly expressed by astrocytes	Differentiation of ICH from IS and mimics	Foerch et al., 2012	39 ICH patients, 163 IS patients, 3 stroke mimics	cohort study <4.5 h from symptom onset
S100B/RAGE	Ligand/receptor which transduces extracellular effects of S100B in astrocytes	Differentiation of ICH from IS	Montaner et al., 2012	139 ICH and 776 IS patients	cohort study <6 h from symptom onset
ApoC-III	Lipoprotein expressed by the liver and found in VLDL but also HDL and LDL particles	Differentiation of ICH from IS and healthy controls	Allard et al., 2004	15 ICH patients, 16 IS patients, 26 healthy controls	case control <6 h from symptom onset
Etiologic markers					
β -amyloid	Protein with significant nonpathological activity, pathologically deposited in the media and adventitia of small- and mid-sized arteries of the cerebral cortex and the leptomeninges in CAA and AD	Diagnosis of Cerebral amyloid angiopathy	Hernandez-Guillamon et al., 2012	29 ICH patients, 21 healthy controls	case control during the first 6 months
Prognostic markers for mortality					
S100B	Calcium-binding protein, marker of glial activation and brain injury in general	Prediction of 1-week mortality Differentiation of ICH from healthy controls	Hu et al., 2010	86 ICH patients, 30 healthy controls	case control at days 1, 2, 3, 5 and 7
Blood glucose level		Prediction of 30-day and long-term mortality	Lee et al., 2010	1387 ICH patients	cohort study on admission for short-term and at 30 days for long-term prognosis
TNF α	Cytokine	Prediction of ICU mortality	Fang et al., 2007	43 ICH patients	cohort study on admission and daily
IL-11	Cytokine	Prediction of overall mortality	Fang et al., 2005	43 ICH patients	cohort study on days 1, 2, 3, 4
CRP	Nonspecific systemic inflammation marker	Prediction of 30-day mortality	Di Napoli et al., 2011	210 ICH patients	cohort study <24 h from symptom onset
D-Dimer	Fibrin degradation product	Prediction of 30-day mortality	Chiu et al., 2012	170 ICH patients	cohort study at 24 h from symptom onset
HSP-70	heat shock protein 70	Prediction of ICU mortality	Fang et al., 2007	43 ICH patients	cohort study on admission and daily
Microparticles	Small membrane vesicles with procoagulant activity	Prediction of 1-week mortality, ICH volume, GCS score	Dong et al., 2011	86 ICH patients, 30 healthy controls	case control on admission and at days 1, 2, 3, 5 and 7
Prognostic markers for functional outcome					
VAP-1/SSAO	Ectoenzyme expressed by endothelial cells, smooth muscle cells and adipocytes, present in plasma resulting from cleavage of the cell surface form	Prediction of neurological improvement at 48 h Differentiation of ICH from healthy controls	Hernandez-Guillamon et al., 2012	66 ICH patients, 58 healthy controls	case control <6 h from symptom onset

Table 1. (continued)

Blood biomarkers in the acute management of ICH						
blood biomarker	description	potential clinical application	study reference	size	study design	time points
s-Fas	Soluble molecules, which spring from alternate splicing of the Fas receptor/ligand apoptosis system	Prediction of perihematomal edema growth	Delgado et al., 2008	78 ICH patients	cohort study	<24 h from symptom onset
S100B	Calcium-binding protein, marker of glial activation and brain injury in general	Prediction of neurological function at discharge	James et al., 2009	28 ICH patients	cohort study	<24 h from symptom onset
MMP-9	Neurotoxic protease, induced by thrombin and blood	Prediction of perihematomal edema and acute worsening	Abilleira et al., 2003	57 ICH patients	cohort study	<24 h from symptom onset
MMP-9	Neurotoxic protease, induced by thrombin and blood	Prediction of early hematoma growth	Silva et al., 2005	183 ICH patients	cohort study	<12 h from symptom onset
BNP	Neurohormone	Prediction of neurological function at discharge	James et al., 2009	28 ICH patients	cohort study	<24 h from symptom onset
TNF α , IL-6	Cytokines	Prediction of early hematoma growth	Silva et al., 2005	183 ICH patients	cohort study	<12 h from symptom onset
TNF α , IL-6, ICAM-1	Cytokines	Prediction of hematoma growth and size Prediction of poor outcome and residual cavity size at 3 months	Castillo et al., 2002	124 ICH patients	cohort study	<24 h from symptom onset
IL-10, Peripheral LcC	Cytokine, inflammation cells	Prediction of rebleeding Prediction of 30-day functional outcome	Wang et al., 2011	59 ICH patients	cohort study	within 2–22 h from symptom onset
HMGB-1	Pro-inflammatory protein, released by micro glia	Prediction of poor outcome at 3 months	Zhou et al., 2010	60 ICH patients, 41 healthy controls	cohort study	<12 h from symptom onset
Peripheral LcC	Part of the inflammatory response	Prediction of poor short- and long-term outcome	Agnihotri et al., 2011	423 ICH patients	cohort study	changes in LcC over the first 72 h from admission
Glutamate	Neurotransmitter of the CNS, toxic in large quantities	Prediction of poor neurologic outcome and residual cavity size at 3 months	Castillo et al., 2002	124 ICH patients	cohort study	<24 h from symptom onset
Ferritin	Free iron released after erythrocyte lysis	Prediction of poor outcome	Perez de la Ossa et al., 2010	92 ICH patients	cohort study	<12 h, at 24 and 72 h from symptom onset
LDL cholesterol	Low density lipoprotein cholesterol	Prediction of hematoma growth, early neurological deterioration and 3-month mortality	Rodriguez-Luna et al., 2011	108 ICH patients	cohort study	<6 h from symptom onset
Fibrinogen	Acute phase protein participating in clot formation	Prediction of early neurologic deterioration	Leira et al., 2004	266 ICH patients	cohort study	<12 h from symptom onset
Factor XIII	Factor agent of blood clot formation	Prediction of hematoma growth >33%	Marti-Fabregas et al., 2010	90 ICH patients	cohort study	<6 h from symptom onset

Table 1. (continued)

Blood biomarkers in the acute management of ICH						
blood biomarker	description	potential clinical application	study reference	size	study design	time points
Copeptin	C-terminal of pro-vasopressin, an agent of stress response	Prediction of hematoma volume Prediction of poor functional outcome at 3 months Prediction of 30-day mortality	Zweifel et al., 2010	40 ICH patients	cohort study	<72 h from symptom onset
Copeptin	C-terminal of pro-vasopressin, an agent of stress response	Prediction of 1-year mortality Prediction of poor outcome at 1 year Prediction of early neurologic deterioration	Zhang et al., 2012	89 ICH patients, 50 healthy controls	cohort study	<6 h from symptom onset
Prognostic markers for good functional outcome						
CD34+ pro-genitor cells	Bone marrow-derived progenitor cells	Prediction of good functional outcome at 3 months	Sobrino et al., 2011	32 ICH patients	cohort study	<12 h from symptom onset and after 7±1d
VEGF, Ang-1, G-CSF	Growth factors in angiogenesis	Prediction of good functional outcome and reduced residual cavity size at 3 months	Sobrino et al., 2009	95 ICH patients	cohort study	at 24±6 and 72±24 h

matrix [41, 42]. On the other hand, MMP-9 contributes in beneficial ways by promoting angiogenesis, remodeling, cell migration, and phagocytosis [43].

In a small cohort study, MMP-9 was found to be elevated after ICH [44]. In cases of deep bleeding, MMP-9 is significantly correlated with edema size and neurological worsening. Another larger cohort study found high MMP-9 levels correlate with a percentage of hematoma growth ($r = 0.64$) [45].

MMP-9 seems to play an important role in complications after ICH [46]. Its role as a predictor of hematoma growth needs validation in larger studies. If validated, MMP-9 may play a role in selecting patients for trials investigating hemicraniectomy in ICH.

BNP

B-type natriuretic peptide (BNP) is a hormone secreted by the smooth muscle cells of the heart when stretched [47]. Recent research [48] suggests that BNP plays a role in disease progression of neuronal injuries including ICH.

In a small cohort study, BNP levels correlated with the ICH score ($r = 0.42$) and independently predicted outcome at discharge [49].

The mechanisms by which endogenous high BNP levels are related to disease progression after ICH are not yet understood and require further research.

VAP-1/SSAO

Vascular adhesion protein-1 (VAP-1) belongs to the semicarbazide-sensitive amine oxidase (SSAO) family, enzymes involved in the process of immune cell migration, and found either on the cell surface or soluble in serum.

In ICH patients, low VAP-1 independently predicted neurological improvement after 48 h ($OR = 6.8$) after accounting for baseline stroke characteristics [50]. This supports the finding that inhibition of VAP-1 activity in mice reduced brain cell destruction [51].

VAP-1 shows potential as an outcome predictor, which needs to be verified in future studies also including stroke mimics. Moreover, comparing VAP-1 activities with other important predictors of outcome, such as ICH volume and early hematoma growth, are required.

s-Fas

Soluble Fas (s-Fas) are variant molecular splicing products of the Fas-receptor/ligand apoptosis signaling pathway, and they belong to the tumor necrosis factor (TNF) receptor family. Some s-Fas molecules were found to be potential inhibitors of the Fas-apoptosis system, and appear to correlate with neuronal cell death [52].

It was shown that s-Fas is significantly lower in ICH patients than in healthy controls, when blood is withdrawn within 24 h from symptom onset. Moreover, low baseline s-Fas proved to be an independent predictor for perihematoma edema growth (OR = 0.125) [53]. Administration of s-Fas [54] inhibits the Fas-receptor/ligand system in a cell model [55], reducing neuronal damage.

These findings should further be elucidated, especially the role of the Fas system in cell death after ICH.

S100B

By inducing neuronal death, S100B stimulates inflammatory stress as well as dispersal of interleukins (IL-1 [56], IL-6) and TNF α [57] and therefore might correlate with disease progression.

In a small case-control study, plasma S100B was found to be significantly increased from symptom onset until day 3 in patients with ICH compared to healthy controls. It was an independent predictor for 1-week mortality [58]. Similarly, another study showed S100B to be an independent predictor for neurological functional outcome at discharge in a small cohort of ICH patients [49]. The predictive value of S100B alone (AUC = 0.88) was slightly lower compared to areas under the curve of GCS scores (AUC = 0.94) and ICH volume (AUC = 0.93) [58].

There is a potential use of S100B as an outcome BBM but it needs verification in larger prospective cohort studies. Moreover, if S100B does not provide incremental value for prognostication in ICH, it might not find its way into clinical routine.

Blood Glucose Level

A large-scale cohort study showed a significant relationship between blood glucose levels (BGL) on admission and 30-day mortality. Even after excluding patients with diabetes, BGL proved an independent risk factor for death (adjusted HR = 1.10, 95% CI 1.01–1.19). BGL re-measured at 30 days revealed increase in long-term mortality with each glucose quartile [59].

These results imply the importance for early BGL monitoring in ICH patients, but need to be taken carefully as management of glucose-levels has been proven delicate in other critically ill patients [60]. Early intervention studies need to evaluate the ideal range for glucose levels after ICH.

Inflammatory Markers

As part of the inflammatory response, cytokines (TNF α , IL-6, IL-10, and IL-11), acute phase proteins (CRP), immune cells (peripheral leukocytes), ICAM (a

member of the immunoglobulin superfamily), and High-Mobility-Group-Protein B1 (HMGB1, is a pro-inflammatory, DNA-binding protein released by microglia [61]) play variable roles in the progress of ICH, being involved in the rupture of vessels, further bleeding and necrosis, but in the subsequent course also acting to promote recovery. Consequently, many of them correlate with severity of stroke in the acute phase.

TNF α , ICAM-1, IL-6, IL-10, IL-11. In a small cohort, increased plasma TNF α on day 1 was found to predict intensive care unit (ICU) mortality [62]. High TNF α and IL-6 levels both proved independent predictors for early hematoma growth in ICH patients and were associated with increased risk of mortality and poor functional outcome at 3 months [45]. Another cohort study showed that TNF α , IL-6 and intercellular adhesion molecule 1 (ICAM-1) independently correlated with hypodensity on CT at days 3–4. In this study, ICAM-1 was also shown to correlate with poor outcome at 3 months and with residual cavity size [63]. In a prospective study enrolling 59 consecutive ICH patients, IL-10 predicted rebleeding with 75% sensitivity and 72% specificity and independently predicted poor 30-day functional outcome with 73% sensitivity and specificity of 66% [64]. Serum IL-11 was found to be significantly higher in nonsurvivors of a small ICH cohort on days 2–4 [65].

CRP. In a large ICH cohort, elevated blood C-reactive protein (CRP) was independently associated with increased 30-day mortality after ICH and even added an 8% improvement to the accuracy of the Hemphill score model [66].

HMGB-1. In a small case-control study, comparing ICH patients to healthy controls, higher plasma HMGB-1 levels measured within the first 12 h were associated with poor outcome. The study also showed significant correlation of HMGB-1 with levels of IL-6 and TNF α and NIHSS ($r = 0.845$) score at day 10 [67].

Peripheral Leukocyte Count. In a case-control study, the change of peripheral leukocyte count (LcC) was examined by measuring the difference between LcC on admission and the highest LcC in the first 72 h. The study showed significant correlation between LcC and poorer discharge disposition (OR = 1.258) as well as decline in modified Barthel index (MBI) at three months ($r = 0.222$) [3]. Another prospective study showed higher LcC in patients rebleeding within 30 days [64].

The mechanisms of brain tissue damage from blood vessel rupture, disruption of the BBB, edema formation, and activation of the inflammatory response system are not yet completely understood. Inflammation may con-

tribute to neurological damage in the beginning but contribute to recovery in late ICH. Larger cohort study designs are necessary, especially for usage in clinical prognostic decisions, to validate these findings over the long-term as these markers follow a dynamic course.

CD34+ Progenitor Cells, VEGF, Ang-1, G-CSF

CD34+ progenitor cells (CD34) in the blood are circulating ancestors of adult endothelial cells and play an important role in angiogenesis. Vascular endothelial growth factor (VEGF), Angiopoietin 1 (Ang-1) and granulocyte-colony stimulating factor (G-CSF) are growth factors (GF) also participating in neoangiogenesis. Assuming a connection between effective neurovascularization and good recovery from hemorrhage, they were evaluated as potential prognostic markers.

CD34. In blood sampled from 32 ICH patients within 12 h from symptom onset and on day 7, CD34 levels at day 7 but not day 1 positively correlated with good functional outcome (OR = 1.17) and negatively with residual cavity volume at 3 months ($r = -0.607$) [68]. A strong correlation was also found between CD34 levels and GFs at day 7.

VEGF, Ang-1, G-CSF. In a previous cohort study measuring GF levels of 95 patients with primary ICH on day 3, independent associations between VEGF, Ang-1 and G-CSF and neurologic improvement (OR = 11.2; 14.7 and 19.6, respectively) as well as reduced residual cavity size at 3 months were demonstrated [69].

The results show that CD34 and GFs are not markers usable in the acute phase of ICH. But as their increase following ICH may be associated with a better functional outcome, further investigation of long-term dynamics of CD34 and GFs and their role in the healing process is warranted.

Glutamate

Glutamate is an important neurotransmitter of the healthy CNS and is pathologically released in excitotoxic quantities by necrotic astrocytes as they appear in ICH. A correlation with secondary damage after ICH is expected.

Glutamate was shown to independently predict poor neurologic outcome at 3 months and to positively correlate with residual cavity size in a cohort of 124 ICH patients ($r = 0.846$) [63].

As glutamate has been well studied in other contexts, it might offer treatment opportunities after ICH, by inhibition of its excitotoxic signaling pathways. Such speculations and its prognostic value need to be confirmed in larger intervention studies.

Ferritin

Free ferritin in the brain occurs in line with hemolysis of the escaped erythrocytes after ICH. The release of iron is assumed to lead to delayed brain edema formation by harming neuronal tissue via oxidative stress, glutamate release, and inflammatory response [8, 70].

In a prospective study, an independent association between free serum levels of ferritin on admission and poor outcome has been shown (stroke severity, ICH, and edema volumes all $r > 0.60$) [70]. No correlation to other acute phase proteins was found, even after adjustment for signatures for acute inflammation. The authors therefore suggest that free baseline ferritin affects outcome directly, and they propose a link to the therapeutic opportunity of iron chelation, which however needs further investigation.

LDL-Cholesterol

Low-density lipoprotein (LDL) is involved in the transportation of lipid molecules (e.g., cholesterol). Although high levels are a risk factor for cardiovascular events, after stroke, low LDL and total cholesterol levels are associated with unfavorable outcomes. It may be that serum cholesterol is needed for the integrity of vessel walls and that lower levels decrease platelet aggregation thus predisposing for ICH growth [71].

A recent cohort study demonstrated an independent correlation between low LDL levels and hematoma growth at 24 h (OR = 4.24), and between early neurological deterioration (OR = 8.27) and mortality at 3 months (OR = 6.34). In this study, pretreatment with statin did not seem to have an influence [71]. In earlier studies, it was shown that low LDL-cholesterol and low total-cholesterol levels predict a higher risk for mortality after ICH [72–74].

Although LDL seems to be a strong and independent predictor, the study does not provide information on the additive value compared to other prognostic scores. There is a risk for selection bias due to exclusion of patients who were either comatose, who died before follow-up CT, underwent surgery, or were under anticoagulant therapy [71].

Coagulation Markers

D-Dimers are degradation products of fibrinolysis; fibrinogen, an acute phase protein, and Factor XIII participate in blood clot formation. Following ICH their blood concentrations change, as the hemostatic system is activated [75] and might allow prognostic information.

D-Dimers. In a cohort study, high plasma D-Dimer levels proved a risk factor for 30-day mortality with a sensitivity of 70% and a specificity of 60%. Moreover, an association between D-Dimer levels and GCS score, midline shift, and subarachnoid extension of the hemorrhage, but no association with intraventricular involvement was shown [76].

Fibrinogen. Fibrinogen was found to be an independent predictor of age, sex, and time from onset to inclusion and Canadian Stroke Scale for early neurologic deterioration (OR = 5.6) in 266 ICH patients [77].

Factor XIII. One study showed increased Factor XIII activities in patients with growing hematomas, and decreased levels in the nongrowing hematoma group [78].

The studies show several associations between coagulation agents and other outcome predictors. Yet at the best cut-off levels, specificity of D-Dimers was rather moderate. Further research in understanding the role of coagulation-related markers in ICH patients is needed.

Copeptin

Copeptin is the C-terminal part of pro-vasopressin, a neuroendocrine stress marker. As ICH is a major stressor, it activates the neuroendocrine stress axes and since copeptin has been associated with unfavorable outcome in patients with IS, it may also be associated with outcome in ICH patients [79].

Copeptin – measured within 72 h from symptom onset in a small ICH cohort – was found to positively correlate with hematoma volume and negatively with the GCS. Moreover, high levels of copeptin allowed prediction of 30-day mortality (AUC of 0.88) and poor functional outcome at 90 days (AUC of 0.68) [80]. In a larger study, it was shown that copeptin independently predicted 1-year mortality and poor outcome as well as early neurologic deterioration [81].

The studies demonstrate copeptin's potential role as a BBM for risk stratification.

HSP-70

Heat shock protein 70 (HSP-70) is involved in the process of protein folding and helps to protect cells from stressors including hypoxia or temperature, as occur also in the brain tissue of ICH patients [82].

In a small study, HSP-70 levels measured on the first day after ICH were found to be higher in nonsurvivors than in survivors [62].

HSP-70 shows some potential as a prognostic marker, but no additive value of HSP-70 to conventional outcome predictors like the Hemphill grading scale have been shown so far.

Plasma Microparticle Concentrations

Microparticles (MPs) are small membrane particles, released by stressed cells under conditions like apoptosis, chemical, or endotoxin stimulation [83]. They seem to play a role in coagulation [84] as well as inflammation [85], both contributing strongly to neuronal damage after ICH.

Plasma MPs independently predicted 7-day mortality with a 91% sensitivity and 69% specificity. Plasma MP levels during the first week were higher in patients suffering from intraventricular hemorrhage, in patients with lower GCS scores or greater ICH volumes. Moreover, the study showed that increased MP levels correlated with higher plasma levels of acute phase response markers such as CRP, D-Dimers, fibrinogen, and BGL [86].

Plasma MPs are potential BBMs for the severity of early brain injury after ICH. Interest of future studies should be given to the origin of the measured plasma MPs to enhance our understanding of the cell types involved in cerebral injury.

Selection of Most Interesting Candidates

Blood Biomarkers Selecting Patients for Specific Treatment Options

The INTERACT2 study suggests that acute blood pressure-lowering therapies (<140 mm Hg) ameliorate outcome for ICH patients [24]. BBMs with promising results addressing early pre-hospital identification of ICH versus IS are GFAP and to some degree S100B/RAGE. If results can be confirmed in larger samples also including mimics and different settings, these BBMs as point-of-care tools could potentially facilitate early decisions on blood pressure therapy.

It seems interesting to consider whether ICH patients could be further subdivided into groups profiting/not profiting from intensive blood pressure lowering or hemostatic interventions during hospitalization. There are two trials (n = 339 [87] and n = 841 [88]) showing significant reduction of hemorrhage size after the administration of recombinant active factor VII (rFVIIa), but nevertheless fail to demonstrate an overall improved outcome. They propose that patients that were not at risk to experience further expansion of hematoma should have been excluded to result in improved outcomes. Blood levels of several BBMs (e.g., MMP-9, IL-10, see table 2) in ICH show an ability to detect patients at risk for further hemorrhage and might have an importance in selecting patients that would profit from therapies diminishing hematoma growth (table 2).

Table 2. Biomarkers detecting patients suitable for surgery

Blood biomarkers predicting further bleeding	
Low LDL	Associated with hematoma growth at 24 h
High Factor XIII	Found in patients with growing hematomas
High LcC	Associated with rebleeding within 30 days
High TNF α and IL-6	Both proved independent predictors for early hematoma growth
High IL-10	Found to predict rebleeding
High MMP-9	Correlates with early hematoma growth

Also surgical interventions (e.g., hemicraniectomy or minimally invasive procedures) require stability of the patient's condition in regard to further bleeding, to possibly show a better overall outcome [26]. The above-mentioned BBMs that depict risk of further hemorrhagic activities (table 2) might be of use to enroll ICH patients to either medical or surgical intervention. Possibly, a combination of those BBMs might be used for the triage, but so far no study has addressed this specific question.

Only randomized controlled trials comparing algorithms with and without the information of BBM values could answer this question.

Selection of Blood Biomarkers Providing Strong Clinical Evidence

Study Design

As the research interest for BBMs in ICH evolved only over the last decade, the concerning studies reside still in an exploratory phase. Many studies are conducted in a case control design, which bears the problem of selection bias per se. Only few cohort studies, and no randomized controlled trials based on BBMs have been conducted.

Study Population

The selection of samples and controls is crucial for the interpretation of the data. Current studies of BBMs in ICH often used healthy controls for comparison or they excluded clinically important comorbidities from samples and controls, which most probable – if included – would impair the results of the discussed markers. It is therefore remarkable, that the ICH sample for VAP-1/SSAO didn't exclude systemic diseases (i.e., severe liver disease), although comparing to healthy controls. The marker ApoC-III, on the other hand, is the only case control study, that included orthopedic patients as controls (without any known peripheral/central nervous system condition), and not healthy subjects. The only study differentiating stroke mimics is the cohort for GFAP.

Sample Size

Also sample size is an important issue and there is clearly need for verification of the results of most discussed BBMs in larger studies. Only few markers were studied in rather large samples, that is, BGL (cohort of 1387 ICH patients), S100B/RAGE (cohort of 139 ICH and 776 IS cases), peripheral LcC (cohort study with 423 ICH patients), fibrinogen (cohort study with 266 ICH patients), CRP (cohort study with 210 ICH cases), and GFAP (cohort study with 39 ICH, 163 IS, 3 mimics). However, validation studies in larger multi-center settings are necessary also for these BBMs.

Time from Symptom Onset

When interpreting the results, it is worth taking into account the particular time of blood withdrawal and the time from symptom onset; both these times should be predefined, as BBMs might follow a dynamic change, therefore altering information or losing comparability over time. BBMs that were measured at multiple predefined time points are S100B, TNF α , IL-11, HSP-70, microparticles, peripheral LcC, ferritin, and GFs, and therefore, allow a more detailed interpretation of the predictive or diagnostic values at given time-points.

Additional Value and Comparison to Clinical Standards

A new prognostic BBM generally needs to provide additional information to established clinical features like the GCS, the ICH score or imaging data. S100B for example shows a predictive value for 1-week-mortality, that lies slightly (on a not significant level) below the values of the GCS score and ICH volume [58]. An integration to show the additive value to such clinical signs would be interesting, as it has been done for the panel of S100B/RAGE to distinguish between patients suffering from ICH versus IS [35]. In this study, an additive value of the panel to the AUC when added to a model using only clinical signs was significant (AUC 0.84 vs. AUC 0.77). Also HSP-70 was tested in terms of its additive value to conventional outcome predictors, unfortunately with no significant result [62].

Blood Biomarkers Interestingly Linked to Pathophysiology of ICH

As a BBM, copeptin – the C-terminal of pro-vasopressin – correlates negatively with the GCS, and positively with poor functional outcome, mortality, and hematoma size. A study investigating the inhibition of the arginine vasopressin (AVP) V1 receptor in mice after ICH, could thereby demonstrate a significant reduction in cerebral

edema formation and significantly less neurobehavioral deficit in the mice treated with the competitive receptor antagonist [89]. They suggest AVP might be a key player of water homeostasis in the brain. Also in a mouse model, VAP-1 (associated with bad outcome in humans) inhibition was shown to reduce brain cell destruction, most likely because it precludes leukocytes from migrating into the injured area, thereby decreasing edema formation [51]. Also s-Fas administration reduced cell-death in a cell model, suggesting a potential therapeutic application of s-Fas [55]. Similarly, another study in rats demonstrated a neuroprotective effect of deferoxamine, an iron chelator, in reducing post ICH edema and neurological injury [90], which underlines the aggravating role of ferritin in disease progression after ICH, suggesting a potential treatment opportunity of iron chelation in humans. As a BBM, BNP correlates with the ICH score. A recent study showed, that its administration in mice after traumatic brain injury or ICH significantly improved functional outcome [91]. In the mice treated with BNP, inflammatory markers (TNF α , IL-6) decreased, microglia was activated, and neuronal damage was reduced, whereas functional performance and cerebral blood flow were increased [91]. It might be that in more severe ICH cases more BNP is released to improve the recovery, but that the chances of recovery are low due to the initial severity of the ICH. How to translate the effect of BNP in the recovery after ICH in mice to human beings where elevated BNP levels are associated with unfavorable outcome is, however, not yet clear. Such additional results from animal or cell models can add valuable patho-mechanistic information, and inspire further research hypothesis.

Conclusions

Different approaches and new research, ranging from genomics over proteomics to classical hypothesis-based biomarker identification, on which we focused in this review, suggest novel BBMs. Novel BBMs that correlate with different ICH-related processes might at some point improve the management and outcome of ICH patients. We outlined how each agent is linked to clinical assessment, therefore potentially advancing the current tools for diagnosis, therapy monitoring, risk stratification, or intervention. Nevertheless, for implementation into clinical routine, larger, well-designed prospective studies will be needed to validate findings and prove utility and benefit of the cited BBMs in addition to available treatment protocols and adjuvants like imaging or clinical scores. In this stage of research where large validation studies are needed, effectiveness might be improved by international cooperation and shared resources, such as those provided by the consortium on stroke biomarker research (<http://stroke-biomarkers.com/page.php?title=Resources>).

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